The anterior pituitary gland: Lessons from livestock

C.G. Scanes [a,b,*], S. Jeftinija [c], A. Glavaski-Joksimovic [d],
J. Proudman [e], C. Arámburo [f], L.L. Anderson [b,d]

[a] *Department of Poultry Science, Mississippi State University, 617 Allen Hall,
P.O. Box 6343, MS 39762, USA*
[b] *Department of Basic Science, Mississippi State University, MS 39762, USA*
[c] *Department of Animal Science, Department of Biomedical Sciences,
Iowa State University, Ames, IA 50011, USA*
[d] *Department of Biomedical Sciences, Iowa State University, Ames, IA 50011, USA*
[e] *Biotechnology and Germplasm Laboratory, Agricultural Research Service,
US Department of Agriculture, Beltsville, MD 20705, USA*
[f] *Department of Cellular and Molecular Neurobiology, Institute of Neurobiology,
Universidad Nacional Autónoma de México, Campus UNAM-UAQ,
Juriquilla, Querétaro, México*

Abstract

There has been extensive research of the anterior pituitary gland of livestock and poultry due to the economic (agricultural) importance of physiological processes controlled by it including reproduction, growth, lactation and stress. Moreover, farm animals can be biomedical models or useful in evolutionary/ecological research. There are for multiple sites of control of the secretion of anterior pituitary hormones. These include the potential for independent control of proliferation, differentiation, de-differentiation and/or inter-conversion cell death, expression and translation, post-translational modification (potentially generating multiple isoforms with potentially different biological activities), release with or without a specific binding protein and intra-cellular catabolism (proteolysis) of pituitary hormones. Multiple hypothalamic hypophysiotropic peptides (which may also be produced peripherally, e.g. ghrelin) influence the secretion of the anterior pituitary hormones. There is also feedback for hormones from the target endocrine glands. These control mechanisms show broadly a consistency across species and life stages; however, there are some marked differences. Examples from growth hormone, prolactin, follicle stimulating hormone and luteinizing

* Corresponding author. Tel.: +1 662 325 3570; fax: +1 662 325 8028.
  E-mail address: scanes@research.msstate.edu (C.G. Scanes).

hormone will be considered. In addition, attention will be focused on areas that have been neglected including the role of stellate cells, multiple sub-types of the major adenohypophyseal cells, functional zonation within the anterior pituitary and the role of multiple secretagogues for single hormones.

1. Introduction

This review will consider the structure of the anterior pituitary gland, the control of the secretion of anterior pituitary hormone and the role of isoforms of these hormones. Attention will be focused on areas where livestock and poultry show specific advantages (see Section 5) and also to arenas that have until recently been neglected.

2. Cells in the anterior pituitary gland

The anterior pituitary gland is composed of a mixture of secretory cells together with non-secretory folliculo-stellate cells (FS). In sheep and horses, this profile is observed in both fetal development and post-natal growth [1,2]. In chickens, FS are not detected until post-hatching [3]. It is likely that sub-types of the major cells (corticotrophs, gonadotrophs, lactotrophs, somatotrophs and thyrotrophs together with FS) exist with different functional characteristics. The possibility of there being zonation/anatomical separation of the cell sub-types has received relatively little attention in livestock species. Zonation would facilitate "point to point" control with specific peptides released from areas within the median eminence and passing via designated portal blood vessels to stimulate specific secretory cells.

2.1. Secretory cells

The anterior pituitary gland is "classically" considered to be composed of the following secretory cells: corticotrophs [producing adenocorticotropic hormone (ACTH)]; gonadotrophs [producing luteinizing hormone (LH) and follicle stimulating hormone (FSH)], lactotrophs or mammotroph [producing prolactin]; somatotrophs [producing growth hormone (GH)]; and thyrotrophs [producing thyrotropin (TSH)]. This situation broadly pertains to livestock and poultry but with a number of exceptions, some of which will be addressed.

2.1.1. Gonadotrophs

There is frequently the assumption that gonadotrophs produce both LH and FSH. This is not necessarily the case. In poultry, there are at least two distinct populations of gonadotrophs, producing, respectively, LH and FSH [4] (discussed in more detail by Berghman in this volume). Moreover, these have distinctly different embryonic origens [5]. In sheep, sub-populations of gonadotrophs exit, some containing both LH and FSH and other

only LH [6]. It is not known whether different sub-populations of gonadotrophs produce specific isoforms of LH and/or FSH.

2.1.2. Lactotrophs and somatotrophs

In addition to the somatotrophs and lactotrophs, there is another cell-type in the anterior pituitary gland, the somatolactotroph or somatomammotroph. This synthesizes both GH and prolactin. This cell-type can be an intermediate in the conversion of somatotrophs to lactotrophs for instance in turkeys when starting to exhibit incubation behavior [7,8] or in the opposite direction at the cessation of incubation [9]. It is not clear the extent the release of GH and prolactin can be independently controlled from somatolactotrophs.

The control of the differentiation of somatotrophs has been extensively examined using chick embryo pituitary cells (discussed in detailed by Porter and colleagues elsewhere in this volume). There are marked changes in the number of somatotrophs in the anterior pituitary gland during development of the chick embryo and during post-hatching growth [10–13]. Moreover, the somatotrophs undergo changes with increases in secretory granules [13]. The percentage of reaches a plateau of ∼40% of the cells in the caudal lobe of the anterior pituitary gland which is maintained despite the continued growth of the anterior pituitary gland [13]. In adult turkeys, there are increases in the number and proliferation of somatotrophs following the termination of incubating behavior when the number of lactotroph is declining [9]. These studies would suggest a "set point" for the number/proportion of somatotrophs and a mechanism(s) controlling somatotroph number/percentage. Moreover, it would also provide support for GH having importance in late growth and in the adult.

It may be questioned whether different sub-types of somatotrophs exist and are stimulated by different secretagogues. Multiple hypothalamic peptides stimulate GH release from the somatotroph. These include the following: GH releasing hormone (GHRH), ghrelin and pituitary adenylate cyclase activating peptide (PACAP) acting, respectively, via GHRH receptors, the GH secretagogue (GHS) receptor (GHSR) and PACAP receptor and increasing intra-cellular calcium (see Table 1) (e.g. [14]). In addition, other peptides increase intra-cellular calcium in somatotrophs. These include leptin (pigs and chickens), motilin (pigs) and thyrotropin releasing hormone (chicken but not pigs). What is interesting is that not all somatotrophs respond to all secretagogues (see Table 1). This might support that the existence of different sub-types of somatotrophs responding

Table 1

Percentage of porcine and chicken somatotropes (defined based on ability of GHRH to elicit an increase in the intra-cellular calcium concentration) responding to various other secretagogues by increase in the intra-cellular calcium concentration

	Porcine	Chicken
GHRH, 10 μM	100[a]	100[a]
PACAP, 1 μM	96	85
TRH, 1 μM	N.A.	73
Ghrelin, 1 μM	98	12
Leptin	54	58

[a] By definition.

to different hypothalamic secretagogues. It is possible that different isoforms of GH or prolactin are produced by various sub-populations of somatotrophs, lactotrophs and/or somatolactotrophs.

2.2. Folliculo-stellate cells (FS)

There is increasing interest in the FS exerting a paracrine control of secretory cells in the anterior pituitary gland. However, this has not received much attention in livestock species as yet. The presence of G-100 protein is frequently used to distinguish FS. Glial fibrillary acidic protein is a marker for astrocytes. However, this protein is not detected in FS in the anterior pituitary gland in some studies (e.g. cattle [15]) but is in others (rat [16]). The FS exert a paracrine effect on the secretory cells based on rodent studies (reviewed [17]). For example, growth of lactotrophs can stimulated by indirectly by transforming growth factor-beta3 acting on FS which in turn release fibroblast growth factor [18]. FS also influence hormone release from secretory cells; mediating effects of steroids on prolactin release (reviewed [19]). Similarly, interferon-gamma inhibits prolactin release with the effect mediated by FS [20]. In addition, FS produce interleukin 6 in response to calcitonin [21]. There is little work on the role of FS in pituitary functioning in livestock species except that bovine FS have been shown to have β-adrenergic receptors coupled to adenylate cyclase [22] and to produce vascular endothelial growth factor and follistatin (Gospodarowicz and Lau [23]).

There is presently untapped potential to use of the pituitary FS in livestock for biomedical models. For instance, the FS of cattle have been employed as models for the proteome of FS with metallothionein I–II immunoreactivity detected recently [24]. Moreover, there appears to be a link between FS and aging based on studies in the pig. During aging, there are marked increases in colloid filled follicles in the anterior pituitary gland [25,26]. The FS, containing G-100 protein, surround the colloid filled follicles and are located in close association with lactotrophs and gonadotrophs [25,26]. It is reasonable, therefore, to propose the pig pituitary and its FS as a model for endocrine aging. Moreover, poultry models may be useful in determining the role of FS. In the chicken, folliculo-stellate cells, as detected by S-100 immunoreactivity, have been reported in the both the cephalic and caudal lobes of the anterior pituitary gland [27]. The FS are in contact with secretory cells that produce LH, GH, POMC and prolactin [1]. There are changes in FS with physiological state. For instance, FS are not detected in the pituitary of the chick embryo but are apparent by 4 weeks of age [1]. Moreover, there are changes in the FS in the caudal lobe of the anterior pituitary following feed withdrawal induction of molt in the hen; the amount of G-100 immunorectivity (area of staining) increasing initially following resumption of lay [27]. Moreover, challenges with *Escherichia coli* have been associated with necrosis of FS in the anterior pituitary gland [28].

3. Control of secretion of anterior pituitary hormones

There is the potential for multiple sites of control of the secretion of anterior pituitary hormones. These include the potential for independent control of the following: (1) proliferation, differentiation, de-differentiation and/or inter-conversion, cell death of specific populations of secretory cells; (2) expression and translation; (3) post-translational

modification (potentially generating multiple isoforms); (4) release with or without a specific binding protein and (5) intra-cellular catabolism (proteolysis) of pituitary hormones.

Geoffrey Harris conceived the physiological system by which the hypothalamus controls the release of hormones from the anterior pituitary gland with releasing factors from the median eminence passing through the hypophyseal portal blood vessels to the secretory cells (reviewed [29]). The concept was essentially that a single releasing factor or hormone acts in an endocrine manner to elicit release of a hormone with the response proportional to the dose. It is increasingly clear that the is secretion of anterior pituitary hormones under control of the following mechanisms:

1. Multiple hypothalamic releasing hormones that may act on secretory cells in specific zones of the anterior pituitary gland;
2. Paracrine factors within the anterior pituitary gland including the putative influence of the FS (see discussion of FS and excitory amino-acids);
3. Peripheral hormones/factors acting directly on the secretory cells or FS.

The latter includes hormones from target tissues that exert feedback effects both at the level of the hypothalamus and anterior pituitary gland (negative feedback: sex steroids on LH and FSH release, inhibin on FSH release, triiodothyronine on TSH release, insulin-like growth factor-1 on GH release and glucocorticoids on ACTH release). In addition, hormone secretion from secretory cells is stimulated by positive feedback acting at the level of the hypothalamus (e.g. in female mammals, estradiol and, in poultry, progesterone stimulating the pre-ovulatory LH surge). In addition to the feedback effects, there are peptides that are produced by peripheral tissues and influence hormone secretion from the anterior pituitary gland. These may or may not be present in the hypothalamus and/or released into the portal blood vessels. Examples of such peptides that influence the release of GH include somatostatin produced by the pancreas and gastro-intestinal tract, ghrelin produced by the stomach, GHRH produced by the placenta and leptin produced by the adipose tissue and liver.

3.1. Peptides

While initially there was the assumption none releasing factor for each adenohyophyseal hormone, the situation is becoming increasingly complex. The release of LH, TSH and ACTH are, respectively, stimulated by gonadotropin releasing hormone (GnRH), thyrotropin releasing hormone (TRH) and corticotropin releasing hormone (CRH). The situation with FSH is problematic. The release of both LH and FSH is stimulated by GnRH in mammals (reviewed see [30,67]). The lamprey GnRH III (lGnRh III) has been proposed as the endogenous specific FSH releasing factor ([31,32], also reviewed [33]) and appears to act in that way in cattle [34]. There are three decapeptide releasing hormones for LH found in the avian hypothalamus; namely cGnRH I, cGnRH II and lGnRh III [35–37,8]. We recently examine compared the ability of the three GnRH peptides to evoke release of LH and FSH in adult male chickens (broiler breeders). Markedly increased circulating concentrations of LH were observed with challenges with cGnRH I or II. These peptides also increased the circulating concentration of FSH but the response was much less robust.

Table 2
Immunological and biological activities of turkey LH isoform (weights adjusted for protein content)

Isoform	Immunological (RIA) potency[a]	Biological activity		
		Progesterone synthesis in vitro[a,b]	Ovulation induction in vivo[c]	
			Low dose	High dose
1	220	12.3	0/2 (30 μg)	2/2 (116 μg)
2	137	8.0	0/2 (34 μg)	1/2 (67 μg)
3	137	4.1	0/2 (49 μg)	2/2 (96 μg)
4	110	4.4	0/2 (36 μg)	2/2 (72 μg)
5	110	1.8	1/2 (15 μg)	2/2 (30 μg)
6	73	N.A.	0/2 (54 μg)	2/2 (222 μg)

N.A., not available.

[a] Potency is expressed as a percentage relative to a turkey LH standard at 50% B/B_0 for RIA and ED_{50} for bioassay (B221B).

[b] Assayed based on the in vitro chicken granulosa cell assay using progesterone production as the end point [2] (F. Hertelendy and J. Proudman, unpublished observations).

[c] Data is expressed as the number of turkey hens exhibiting premature ovulation in response to stimulus. Ovine LH failed to induce ovulation at 500, 1000 and 2000 μg doses (data from H. Opel and J. Proudman, unpublished).

In contrast, lGnRH III evoked only a small response in circulating concentrations of LH and no change in those of FSH.

Despite the supposedly straight-forward roles for both GH and prolactin, there are multiple releasing hormones for each. Control for prolactin release is via a series of releasing factors including vasoactive intestinal peptide (VIP)(+), PACAP(+), TRH(+) and dopamine(−). The releasing hormones for GH include GHRH, ghrelin, PACAP and TRH (all stimulatory) and somatostatin (inhibitory) (see Table 2). It might be questioned on what basis do we call the peptide, GHRH, as ghrelin or PACAP also stimulate GH release? Similarly, on what basis do we call TRH as the peptide also stimulates release of PRL (mammals) and GH (birds) and CRH stimulates TSH release in birds?

3.2. Excitatory amino-acids

There is substantial evidence that excitatory amino-acids such as *N*-methyl-D-asparate (NMDA) stimulate the release of anterior pituitary hormones in rodent models. There is, however, a dearth of information on the direct effects of these in livestock. Consider the case of GH, NMDA increases its release from rat pituitary cells (e.g. [38,39,11,40–42]). Similarly, in vivo studies in livestock indicate that NMDA increases GH secretion (cattle [43]; pigs [44,45]; horses [46]). It is not known whether these effects in livestock reflect direct effects on the somatotrophs or indirect effects via the hypothalamus. There is, however, no information in the direct effects of NMDA on somatotrophs of any livestock species. Moreover, there are no reports of the effects of NMDA influencing the release of pituitary hormone in poultry except that NMDA has been observed to influence the release of GnRH from hypothalamic tissue in vitro [47]. It is not clear whether the excitatory amino-acids that influence GH release originate in the hypothalamus and/or cells within the anterior pituitary gland, for instance, the FS.

4. Isoforms to proteomics

There has been tremendous progress made in our understanding of gene expression in the anterior pituitary gland [48]. In the near future, there will be considerable attention focused on the pituitary proteome, particularly at the individual cell level. A substantial number of biologically active peptides have been and it is predicted will be found in the anterior pituitary gland. For instance, pancreastatin has been found in the pig pituitary gland [12]. In contrast to the strong interest in the biomedical significance of post-translational isoforms of anterior pituitary hormones, these isoforms in livestock and poultry have received less attention. Post-translational isoforms of LH exist with, for instance chicken and porcine LH [49,13]. These have different biological activities (for chicken LH, see Table 2). Similarly, there is evidence of different isoforms of FSH with different biological activities that are released in livestock mammals [50,51]; also see review [52] and of the α sub-unit of LH, FSH and TSH [53]. There are multiple post-translational isoforms of prolactin. These include the following: *glycosylated* (e.g. ovine [54], porcine [55], turkey [56]), *phosphorylated* (e.g. bovine [68]; *cleaved* prolactin [69] and *cleaved and reduced fragments* of prolactin (e.g. rat 16 kDa [57]) These isoforms can have different biological activities. For instance, glycosylated prolactin has reduced pigeon crop assay activity [58]. Phosphorylated prolactin exhibits different activities than non-phosphorylated prolactin, not being luteotropic but also antagonizing the mitogenic effects of prolactin [59–61]. The 16 kDa rat prolactin has a low prolactin activity [57] but exhibits anti-angiogenic activity ([62]; reviewed [63]). The relative concentrations of different isoforms can exhibit marked changes with physiological state (e.g. glycosylated versus non-glycosylated prolactin in pigs [64,65] and turkeys [6,7]).

A similar situation exists for GH. Using the example of the chicken, GH isoforms include the following: (1) a splicing variant expressed in the eye [66] which analogous to the 20 kDa human GH and (2) post-translationally modified isoforms, e.g. phosphorylated [3,4], glycosylated [9], dimeric and other oligomeric forms [4], proteolytically cleaved and both cleaved and reduced to yield a 15 kDa fragment [5]. Monomeric GH binds to the GH receptor [4,70] and is biologically active—stimulating hepatic IGF-1 release and lipolysis [70]. The glycosylated GH is biologically active but has a different clearance rate [10]. No GH receptor binding activity is seen with dimer GH, other oligomers of GH or the 15 kDa fragment of GH [4,5]. However, the 15 kDa GH isomer/fragment has angiogenic activity as indicated by stimulation of proliferation of endothelial cells [5]. It might be noted that the profile of GH isoforms in the pituitary exhibit changes during growth and development with more 15 kDa in the embryonic pituitary gland [4].

5. Advantages of domestic animals in pituitary research

There are advantages of using livestock and poultry for endocrine research. These include the following: (1) the agricultural/economic importance and hence potential funding (government and private industry) of the reproduction, growth, milk production (mammogenesis, lactation, etc.) and responses to stress; (2) availability of disparate genetic breeds, varieties and lines with known genetics (including polymorphisms in candidate genes, e.g. SNPS); (3) the established body of knowledge on interactions between genotype, phenotype and

environment; and (4) that livestock and poultry represent multiple evolutionarily lines separated by about 200 million years ago. Disadvantages of using livestock include the lack of "knock-out models" and the difficulty in maintaining livestock in many biomedical facilities.

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